Temperature Change Does Not Affect Force between Single Actin Filaments and HMM from Rabbit Muscles

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ABSTRACT The temperature dependence of sliding force, velocity, and unbinding force was studied on actin filaments when they were placed on heavy meromyosin (HMM) attached to a glass surface. A fluorescently labeled actin filament was attached to the gelsolin-coated surface of a 1-μm polystyrene bead. The bead was trapped by optical tweezers, and HMM–actin interaction was performed at 20–35°C to examine whether force is altered by the temperature change. Our experiments demonstrate that sliding force increased moderately with temperature ($Q_{10} = 1.6 \pm 0.2$, $\pm$SEM, $n = 9$), whereas the velocity increased significantly ($Q_{10} = 2.9 \pm 0.4$, $n = 10$). The moderate increase in force is caused by the increased number of available cross-bridges for actin interaction, because the cross-bridge number similarly increased with temperature ($Q_{10} = 1.5 \pm 0.2$, $n = 3$) when measured during rigor induction. We further found that unbinding force measured during the rigor condition did not differ with temperature. These results indicate that the amount of force each cross-bridge generates is fixed, and it does not change with temperature. We found that the above generalization was not modified in the presence of 1 mM MgADP or 8 mM phosphate.

INTRODUCTION

It has been known for some time that myosin cross-bridges generate force by interacting with actin molecules on the thin filament when they are placed in a solution that contains Ca^{2+} and $MgATP^{2-}$. It also has been known that the amount of force generation increases with an increase in the temperature in studies that used mammalian skeletal muscle fibers (Goldman et al., 1987; Zhao and Kawai, 1994; Ranatunga, 1996). To account for this temperature effect, two hypotheses are proposed. One hypothesis assumes that the force per cross-bridge does not change, but the number of force-generating cross-bridges increases with the temperature (Zhao and Kawai, 1994). The other hypothesis assumes that the force per cross-bridge increases with an increase in the temperature, but the number of cross-bridges does not change. In these experiments, force (F) is formulated in the following way:

$$F = \underset{\text{(Fiber)}}{N\sum_i f_i X_i} = \underset{\text{(In vitro motility assay)}}{N f_1 X_1}, \quad (1)$$

where N is the number of active cross-bridges that are involved in cycling. In fiber studies, f_i is the force associated with a cross-bridge at a state i, and X_i is the probability of the cross-bridge at the state i ($0 \le X_i \le 1$). The summation is over the all cross-bridge states (6–7 states were identified in fiber experiments) that are arranged in parallel in half sarcomere. Eq. 1 is modified from Kawai and Zhao (1993) to allow a change in the number of actively cycling cross-bridges (N). In the first hypothesis, X_i changes with temperature, and in the second hypothesis, f_i changes with temperature.

To obtain an insight on which one of the three parameters in Eq. 1 might change with temperature at the molecular level, we designed experiments using an in vitro motility assay, and studied sliding force between heavy meromyosin (HMM) molecules attached to a glass slide and an actin filament attached to a polystyrene bead that was clamped by a laser trap (optical tweezers). In the in vitro motility system, two cross-bridge states have been recognized. One is the attached state ($i = 1$) that generates force ($f_1 > 0$), and the other is the detached state ($i = 2$) that does not generate force ($f_2 = 0$). The fiber form of Eq. 1 can be applied to the in vitro motility system, if we assume that X_1 is the duty ratio (Howard, 1997) and f_1 is the unitary force. In this case, the product f_1X_1 is the average force/cross-bridge. Quite interestingly, our results demonstrate that, although force (F) increases with temperature, this increase is primarily based on the number of cross-bridges (N), but f_1X_1 remains approximately the same as the temperature is changed in the range 20–35°C. A preliminary account of the present work was reported in a recent Biophysical Society meeting (Kawai et al., 1999).

METHODS

Experimental apparatus

The experimental apparatus used here is the same as reported previously (Miyata et al., 1994, 1995; Nishizaka et al., 1995a; Nishizaka, 1996; Ishiwata, 1998). The entire apparatus is mounted on a pneumatic isolation table (Herz Kogyo KK, Tokyo, Japan). The optical system is based on an inverted microscope (TMD-300, Nikon, Tokyo, Japan). The light from an

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Nd-YLF laser (1053 nm, 1W: Amoco Laser, Naperville, IL) is led through the oil-immersion objective lens (100×, n.a. = 1.3) from down below and traps a polystyrene bead in the flow cell to function as optical tweezers. The trap center of optical tweezers can be moved at a constant rate by controlling a mobile mirror with two DC-servo motors (Optmike-e; Sigma Koki, Hidaka, Japan). The light from a Halogen lamp, filtered at 380–520 nm, illuminates the flow cell, and its image is recorded by the CCD camera. The light from a Hg lamp is filtered at 550 nm and illuminates the flow cell from below. Accompanying fluorescent light (>590 nm) is led into the image intensifier (KS 1381, Video Scope, Washington, DC) before it is recorded by another CCD camera (CCD-72, Dage-MTI, Michigan City, IN). Phase contrast and fluorescence microscope images are combined and monitored on one screen. The combined image is videotaped (Hi8 Video, Sony Corp, Tokyo, Japan) for later analysis.

Flow cell

Both surfaces of a large coverslip (24 × 60 × 0.15 mm) were coated with collodion dissolved in 3-methylbutyl acetate. A small coverslip (20 × 20 × 0.15 mm) was glued to the large coverslip with double-stick tape at two sides. The gap between the two glass surfaces was about 0.1 mm. The total volume of the flow cell was about 25 μl. From one open side, 25 μl of HMM solution (1 μg/ml for rigor, else 60 μg/ml in solution HD) was applied and settled for 1 min to allow the HMM molecules to adsorb on the glass surface. Another 25 μl of HMM solution was applied from the other side. The cell was then washed by 25 μl of an experimental solution (CONT, +P, +ADP, or Rigor; Table 1). After 1 min, 25 μl rhodamine-phalloidin-labeled actin filaments in the same experimental solution were flowed in. Actin filaments were attached to polystyrene beads. The two open sides were then sealed by enamel (nail polish), and the flow cell was placed in the experimental apparatus.

Experimental solutions

Solutions used for the present studies are summarized in Table 1. Before mixing solutions, doubly distilled water was depressurized for 20 min by aspirator to minimize dissolved O_2. ATP was added as $Na_2H_2ATP{\cdot}2H_2O$, Pi as $H_{1.5}Na_{1.5}PO_4$, ADP as $KH_2ADP{\cdot}3H_2O$, EGTA as H_4EGTA, and pH was adjusted to 7.40.

Proteins, actin filaments, and polystyrene beads.

G-actin and HMM were purified from rabbit white skeletal muscles as described (Suzuki et al., 1996). Gelsolin was purified from bovine serum (Kurokawa, 1990). Polystyrene beads (1.0-μm diameter; Suzuki et al., 1996) were washed with carbonate and phosphate buffers, and coated with BSA and gelsolin. Actin was polymerized, rhodamine-conjugated phalloidin was bound, then F-actin was attached to polystyrene beads. Gelsolin binds to F-actin and serves as an anchor at the barbed end.

Temperature study

For experiments at 20 and 25°C, the room temperature was equilibrated to the respective temperatures. For experiments at 30 and 35°C, the inverted microscope, including the stage and the flow cell, was enclosed by a Nikon-plexiglass cover and its inside temperature was controlled. The temperature was measured by a thermister attached to the microscope stage near the flow cell, and regulated within ±1°C of the designated experimental temperature. The temperature in the flow cell was also estimated from thermal quenching of the fluorescence from rhodamine-maleimide conjugated to tubulin subunits of microtubule (Kawaguchi and Ishiwata, manuscript in preparation), a method similar to that reported earlier (Kato et al., 1999). For the purpose of Q_{10} calculation, Q_5 was first obtained and averaged, and then the Q_{10} was calculated as the square of Q_5. Q_5 is defined as the ratio of a parameter, which is measured at two temperatures that are 5°C apart.

RESULTS

Force on cross-bridges at different temperatures

A bead to which an actin filament is attached is placed 5 μm above the HMM-coated glass surface, which is moved to the left to align the filament parallel to the surface (Fig. 1 *A*). Then, the bead is lowered within 1.0 μm from the surface and filament–HMM interaction is performed in the presence of ATP (Fig. 1, *B* and *C*). From the video image (30 frames/sec), the displacement of the bead from the trap center is traced against time (Fig. 2). Sliding force is calculated as Force = k ∗ Displacement, where k is the spring constant of the optical trap (k = 0.29 pN/nm). For detecting small force, the spring constant k was attenuated either to 50 or 30% of the value by inserting an appropriate neutral-density filter on the incident Nd-YLF laser beam. The length of actin filament was determined from the video image. Because the amount of force is proportionate to the length of actin filament (Kishino and Yanagida, 1988), the force value is divided by the length value to obtain force per unit length of the actin filament.

Figure 3 represents force plotted against the temperature for three experimental conditions (control, +Pi, +ADP).

TABLE 1 Solution compositions

	ATP (mM)	Pi (mM)	ADP (mM)	$MgCl_2$ (mM)	EGTA (mM)	KCl (mM)	DTT (mM)	Im-HCI (mM)	BSA (mg/ml)	GOC* (+/−)
AB†	–	–	–	4	1	25	–	25	–	−
HD	–	–	–	4	1	25	2	25	–	−
CONT	1	–	–	4	1	25	10	25	0.5	+
+Pi	1	8	–	4	1	8	10	25	0.5	+
+ADP	1	–	1	4	1	25	10	25	0.5	+
Rigor	–	–	–	3	1	28	10	25	0.5	+

*GOC represents 4.5 mg/ml glucose, 0.216 mg/ml glucose oxidase, and 0.036 mg/ml catalase to remove dissolved O_2 to minimize photo bleaching (Harada et al., 1990). In all solutions, pH was adjusted to 7.40.

†AB, actin buffer; HD, diluting solution for HMM; DTT, Dithiothreitol; Im-HCI, Imidazole-HCI.

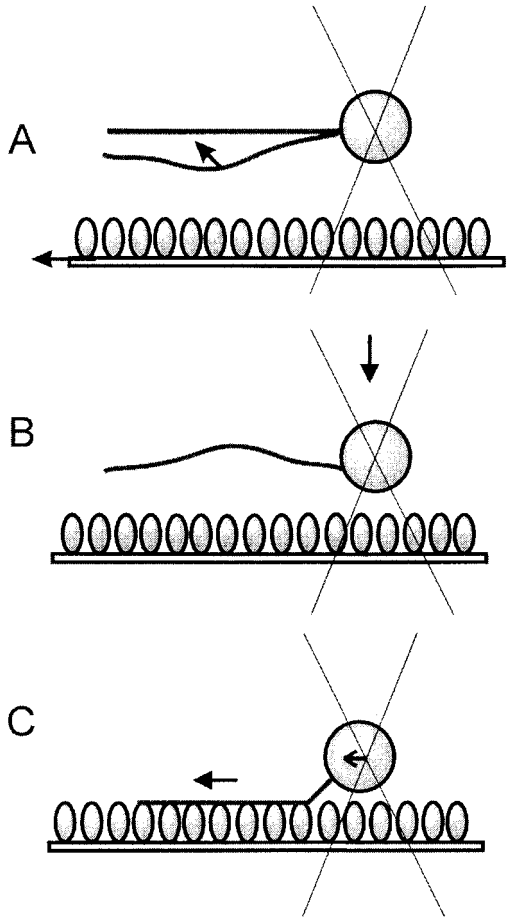

FIGURE 1 The method of measuring force on the single actin filament by optical tweezers. (*A*) An actin filament-attached bead (1 μm in diameter) is placed 5 μm above the HMM-coated glass surface, which is moved to the left (*lower arrow*) to line up the filament in parallel to the glass surface (*upper arrow*). (*B*) The bead is lowered within 1.0 μm from the glass surface (*arrow*). (*C*) The actin filament interacts with HMM to generate leftward force (*larger arrow*). This causes the bead displacement from the trap center indicated by the X mark. The displacement of the bead is measured (*small arrow*) and force is calculated by multiplying the spring constant of the optical tweezers.

Experiments were carried out in the presence of 1 mM ATP (Table 1). For these experiments, 60 μg/ml HMM was used to coat the glass surface. A proportionate relationship between force and the HMM concentration was demonstrated for 0–200 μg/ml HMM (Nishizaka, 1996) under similar experimental conditions. As shown in Fig. 3, a small increase in force was observed as the temperature was increased in the range of 20 to 35°C. This trend was not altered in the presence of 1 mM ADP or 8 mM Pi, except that force was slightly larger in the presence of ADP. From Fig. 3, we calculated Q_{10} for the control condition to be 1.6 $\pm$ 0.2 ($\pm$SEM, $n = 3$), Q_{10} in the presence of 8 mM Pi to be 1.8 $\pm$ 0.4 ($n = 3$), and Q_{10} in the presence of 1 mM ADP to be 1.4 $\pm$ 0.3 ($n = 3$). These values were not significantly different from the control condition. The averaged Q_{10} for all activating conditions tested was 1.6 $\pm$ 0.2 ($n = 9$).

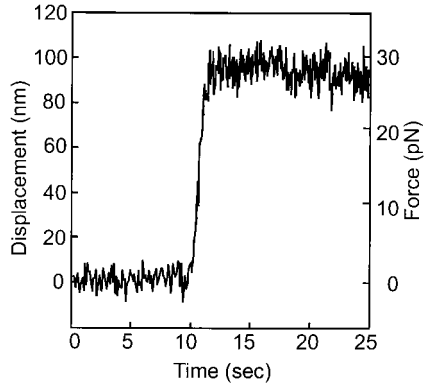

FIGURE 2 The time course to measure force on single actin filament. An actin filament-attached bead is placed within 1.0 μm above the HMM-coated surface, and filament-HMM interaction is performed in the presence of ATP (Fig. 1). The displacement (*left ordinate*) of the bead from the trap center is traced against time at the video rate (30 frames/sec). Sliding force (*right ordinate*) is calculated as Force = $k *$ Displacement, where k is the spring constant of the optical trap ($k = 0.29$ pN/nm). The length of this particular actin filament was 4.8 μm.

Cross-bridge number per unit length of the actin filament

Because the total number of cross-bridges (N in Eq. 1) available for interaction with the actin filament may vary

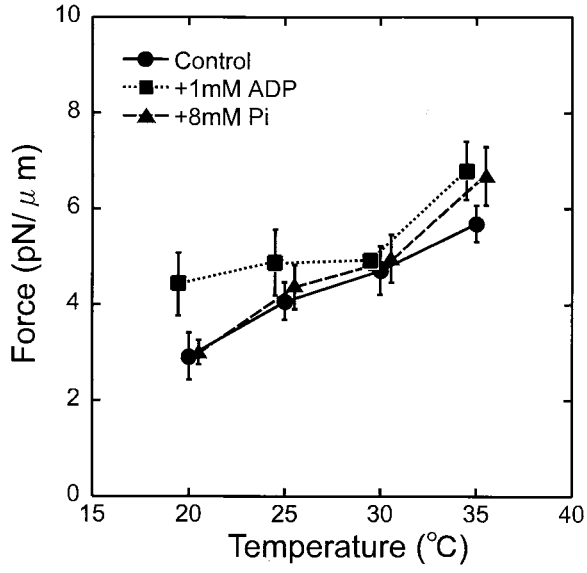

FIGURE 3 Force (pN) per unit length (μm) of actin filament is plotted against the temperature for three experimental conditions (control, +Pi, and +ADP) in the presence of 1 mM ATP. The error bars represent $\pm$SEM. In the control experiment, $n = 4$, 5, 3, and 5 for 20, 25, 30, and 35°C, respectively. In the presence of Pi or ADP, $n = 3$ for 20°C, and $n = 4$ otherwise. Force was measured as shown in Figs. 1 and 2.

depending on the temperature, we counted the cross-bridge number during rigor induction, i.e., in the absence of ATP. For this series of experiments 1 μg/ml HMM was used to coat the glass surface. The method of counting the cross-bridge number and measuring unbinding force in the absence of ATP is depicted in Fig. 4 (see also Fig. 4 of Miyata et al., 1995; Fig. 2a of Nishizaka et al., 1995a; and Fig. 1 of Nishizaka et al., 1995b). The actin filament-attached bead is placed 2–3 μm above the HMM-coated surface, and filament–HMM interaction is performed. This height is necessary to count the cross-bridge number so that the unbound HMM does not rebind to the actin filament. From the video image, the displacement of the bead from the trap center is plotted against time (Fig. 4).

The gap on the trace represents unbinding of a single HMM molecule from the actin filament. The number of gaps is counted and divided by the filament length to obtain the number of cross-bridges per 1 μm of the actin filament. This method determines N in Eq. 1, the number of cross-bridges involved in cycling. A proportionate relationship between the number of cross-bridges and the HMM concentration was demonstrated for 0–15 μg/ml HMM under similar experimental conditions (Nishizaka, 1996, and manuscript in preparation). At a higher concentration, too many cross-bridges are formed, hence, counting the cross-bridge number becomes not possible. The results are plotted in Fig. 5. This figure demonstrates that the number of cross-bridges available for interaction with the actin filament increases with temperature. From Fig. 5, the averaged Q_{10} was calculated to be 1.5 $\pm$ 0.2 (n = 3).

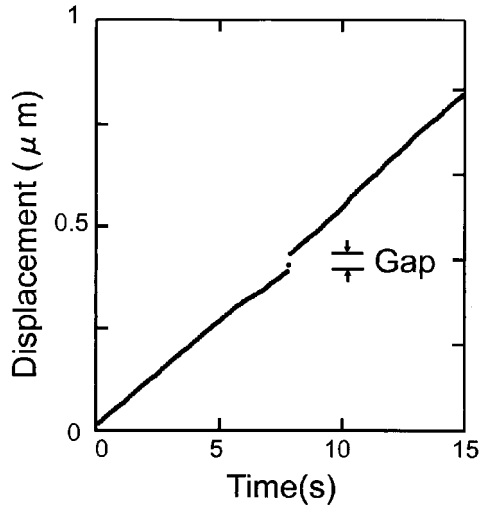

FIGURE 4 The time course to measure the number of cross-bridges and the unbinding force on single HMM molecules in rigor state. An actin filament-attached bead is placed 2–3 μm above the HMM-coated surface, and filament-HMM interaction is performed in the absence of ATP. The displacement of the bead from the trap center is traced against time. The gap on the trace represents unbinding of a single HMM molecule from the actin filament. The number of gaps is counted and divided by the actin filament length to obtain the number of cross-bridges per unit length. The unbinding force is calculated from the gap.

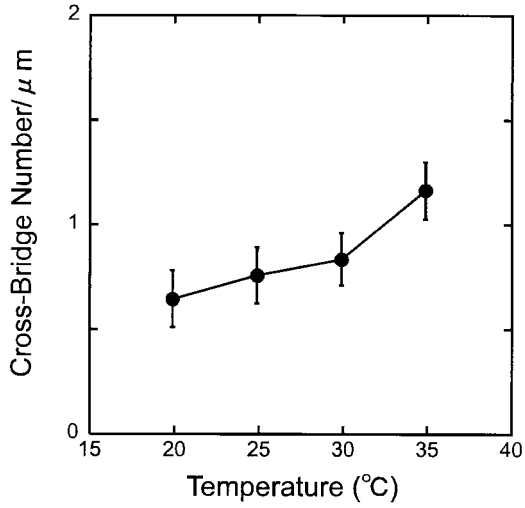

FIGURE 5 The cross-bridge number per unit length (μm) of actin filament in the absence of ATP is plotted against the temperature. The method of counting the cross-bridge number is shown in Fig. 4. The error bars represent $\pm$SEM. n = 3, 4, 4, and 3 for the experiment at 20, 25, 30, and 35°C, respectively.

Corrected force on cross-bridges at different temperatures

Because the number of cross-bridges available for interaction with the actin filament increased with the temperature (Fig. 5), we divided the data of Fig. 3 with the data of Fig. 5, and plotted the results in Fig. 6. The errors in Figs. 3 and 5 were propagated and entered in Fig. 6 in a proper way (see figure legends). Figure 6 represents results from three experimental conditions (control, +1 mM ADP, and +8 mM Pi). Figure 6 demonstrates that the average force per cross-bridge (f_1X_1 in Eq. 1) remained approximately the same as the temperature was changed in the range 20–35°C in three experimental conditions.

Unbinding force

If the force generated by a cross-bridge changes with the temperature, this mechanism implies that a macromolecular change, such as in the shape of the myosin head, occurs when the temperature is elevated. If this is the case, then we expect that "unbinding force" also increases with the temperature. Unbinding force is the force at which a myosin cross-bridge detaches (unbinds) from actin when pulled away by external force, and measured by the "gap" in Fig. 4. The measurement is carried out in the absence of ATP, i.e., when rigor cross-bridges are formed. Unbinding force is plotted against the temperature in Fig. 7. As this figure

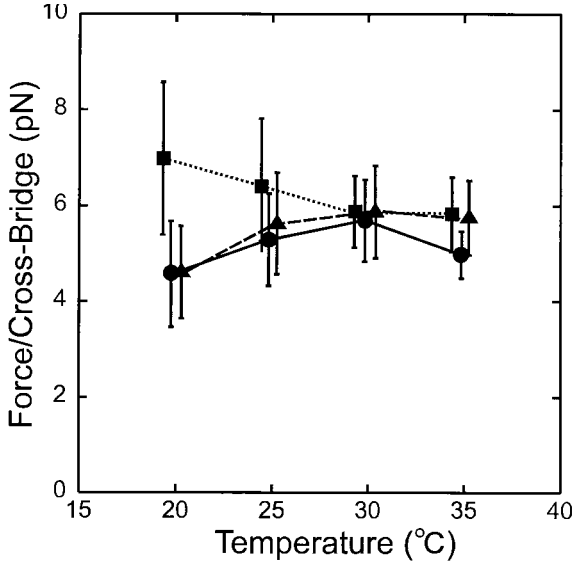

FIGURE 6 Corrected force is plotted against temperature. The data were obtained by dividing the values of force (Fig. 3) with the cross-bridge number (Fig. 5) to compensate for the artifact due to two-dimensional motility assay system, in which the number of HMM molecules within reach to the actin filament changes with the temperature. The same plotting nomenclatures as in Fig. 3 are used. The error bars E_z are calculated by $(E_z/Z)^2 = (E_x/X)^2+(E_y/Y)^2$ where X, Y, Z are the averaged values of Figs. 3, 5, 6, respectively ($Z = X/Y$), and E_x and E_y are SEM of Figs. 3 and 5, respectively.

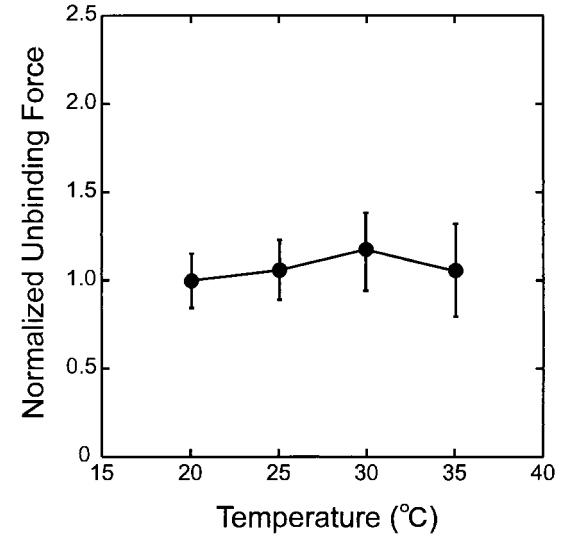

FIGURE 7 Unbinding force in the absence of ATP is plotted against the temperature after normalization at 20°C. The error bars represent ±SEM. n = 14, 17, 12, and 11 for experiments at 20, 25, 30, and 35°C, respectively. For these experiments, 1 μg/ml HMM was used to coat glass surface.

demonstrates, the unbinding force did not change much in the temperature range 20–35°C.

Sliding velocity

Unlike sliding force, the sliding velocity is expected to increase with temperature. To measure the velocity, actin filaments are placed on the HMM-coated surface, and the filaments are allowed to slide over the surface. From the video image (30 frames/sec), the position of the filament is obtained, and the sliding velocity is estimated from the time course in which the translation is linear over 5 μm. The results are plotted in Fig. 8 against the temperature for three experimental conditions (control, +Pi, and +ADP).

The experiments were carried out in the unloaded conditions and in the presence of 1 mM ATP. For this series of experiments, the standard 60 μg/ml HMM was used to coat the glass surface. As shown in Fig. 8, the velocity increased significantly with temperature with the average Q_{10} of 3.0 ± 0.7 (n = 4) in the control condition. The velocity was slightly faster in the presence of 8 mM Pi, and slower in the presence of 1 mM ADP. In this sense, the velocity behaves similar to the apparent rate constant of tension transients. The apparent rate constant $2\pi b$ (the rate constant of delayed tension) is faster in the presence of Pi and slower in the presence of ADP (Kawai and Zhao, 1993). From Fig. 8, Q_{10} in the presence of 8 mM Pi was calculated to be 3.0 ± 1.2 (n = 3), and Q_{10} in the presence of 1 mM ADP was 2.7 ± 0.9 (n = 3). These Q_{10} values were not significantly differ-

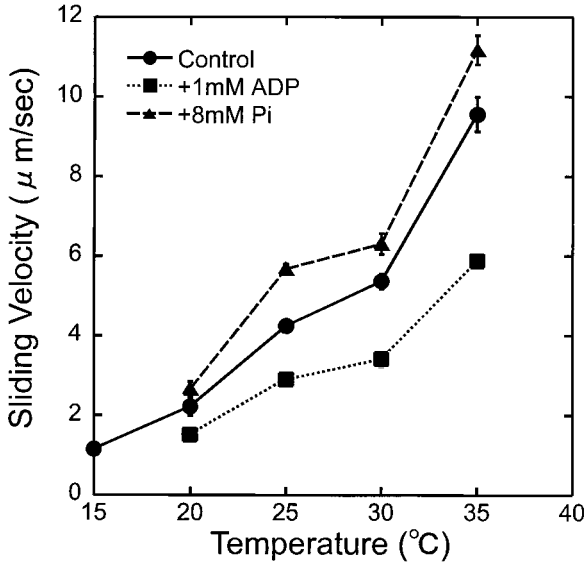

FIGURE 8 Sliding velocity is plotted against the temperature for three experimental conditions (control, +Pi, and +ADP) in the presence of 1 mM ATP. The error bars represent ±SEM. Error bars smaller than the symbol size cannot be seen. In the control experiment, n = 5, 4, 5, and 4 for 20, 25, 30, and 35°C, respectively. In the presence of Pi, n = 4, 3, 4, and 5 for experiments at 20, 25, 30, and 35°C, respectively. In the presence of ADP, n = 4, 5, 4, and 3 for experiments at 20, 25, 30, and 35°C, respectively.

ent from that of the control activating condition. The averaged Q_{10} for all conditions tested was 2.9 ± 0.4 (n = 10).

DISCUSSION

The purpose of the present study was to determine whether the isometric force on a cross-bridge increases with temperature using the in vitro motility assay system. For this purpose, we used synthetic actin filaments attached to polystyrene beads. The actin filaments were visualized by fluorescence as rhodamine-conjugated phalloidin was bound to the filament. HMM was attached to a collodion-coated glass surface. The position of the polystyrene beads were controlled by the laser trap (optical tweezers), and the interaction between HMM molecules and the actin filament was monitored.

We observed an increase in force as the temperature was elevated (Fig. 3; see also Kato et al., 1999). There are two possibilities to account for this increase: the cross-bridge number available for force generation (N in Eq. 1) may increase with temperature, and average force/cross-bridge (f_1X_1 in Eq. 1) may increase with temperature. We determined the cross-bridge number in the absence of ATP (rigor condition), because it is not possible to determine the number in the presence of ATP under the present set up. Interestingly, we found that the cross-bridge number increased with temperature (Fig. 5) just in the same way as force did. This increase may not be a physiological phenomenon, however, because the cross-bridge number is presumably maximized during the rigor induction, hence the number should not change with temperature in a physiological experiment. Therefore, the observed number increase may be specific to the in vitro motility system (Kato et al., 1999). One possibility is that the Brownian motion may release the head portion of HMM that are inadvertently stuck to the glass surface, but it leaves the C-terminus area of HMM attached to the glass surface, so that more HMM molecules become available to interact with the actin filament to generate more force at higher temperatures.

For this reason, we divided isometric force by the cross-bridge number to obtain corrected isometric force (f_1X_1, Fig. 6). We found that the corrected force does not change much with temperature in all three experimental conditions, from which we infer that the average force per cross-bridge does not change much with temperature in the in vitro motility assay system. The corrected force averaged 4.9 pN (range: 4.3–5.5 pN). This is not the unitary force, however, for this force value does not consider a 60× difference in the HMM concentration used in two sets of experiments. To obtain cross-bridge force, the ordinate values of Fig. 6 should be divided by the factor ~60, because the number of cross-bridges available for actomyosin interaction was 60× larger for the experiment of Fig. 3 than for the experiment of Fig. 5. Nishizaka (1996, and manuscript in preparation) demonstrated a proportionate relationship between the cross-bridge number and HMM for up to 15 μg/ml under the rigor condition, but no data are available for concentrations higher than 15 μg/ml. The factor must be >15, but ≤60, because the HMM concentration and the cross-bridge number may be nonlinear in this concentration range. Such nonlinearity is remote, however, because force and the HMM concentration are proportionally related for up to 200 μg/ml HMM under similar experimental conditions (Nishizaka, 1996). Thus, we calculate from our results that the force per cross-bridge (f_1X_1) is somewhere between 0.08 pN and 0.3 pN. The reason why this force value is less than unitary force (f_1) measured between actin and myosin molecules (1–6 pN, Finer et al., 1994; Miyata et al., 1995; Molloy et al., 1995; Ishijima et al., 1996) is because, in our measurement, force is the average over attachment and detachment cycle, whereas the unitary force (f_1) measures force during attachment. It is known that the duty ratio (X_1) is small in the in vitro motility assay system, and it is about 0.1 (Howard, 1997). What is important in this report is our conclusion that the average force per cross-bridge does not change much with temperature.

Although we could not examine the unitary force (f_1) and the duty ratio (X_1) independently, a possibility that both f_1 and X_1 change with temperature in a compensatory manner so that f_1X_1 remains the same is remote, because it is not likely that such compensation takes place at all temperatures we tested, and in all conditions (control, +Pi, and +ADP) we studied (Fig. 6). Furthermore, we measured unbinding force in the absence of ATP and studied the effect of temperature. We found that the unbinding force did not change much with temperature (Fig. 7). From results shown in Figs. 6 and 7, we infer that the HMM molecules do not change their shape much as the temperature is increased. A change in the shape would be necessary if force per cross-bridge or unbinding force were to change with the temperature, because force is presumably a result of the macromolecular architecture of the HMM and actin interrelationship. In this sense, it can be concluded that the result of Fig. 7 (unbinding force does not change with temperature) and the result of Fig. 6 (cross-bridge force does not change with temperature) are consistent.

In experiments using fast-twitch single skeletal muscle fibers, isometric force increases with temperature, and this increase was ascribed to an increase in the number (X_i in Eq. 1) of tension-generating cross-bridges (Zhao and Kawai, 1994), or to an increase in the force (f_i in Eq. 1) supported by an individual cross-bridge (Goldman et al., 1987; Kraft and Brenner, 1997). Both of these mechanisms result in an increase in the average force per cross-bridge (f_iX_i) with temperature. The total number of cross-bridges (N) involved in cycling is assumed not to change with temperature, because maximal activating condition with a saturating Ca^{2+} concentration was used in the fiber experiments. These experiments and mechanisms are primarily based on the temperature range ≤20°C. The effect of temperature is

smaller or absent when temperature is raised over 20°C (Bershitsky and Tsaturyan, 1992; Goldman et al., 1987; Zhao and Kawai, 1994; Ranatunga, 1996). Therefore, our results that the force/cross-bridge does not change much in the temperature range >20°C are consistent with the results on fiber experiments.

Sliding velocity is expected to increase with the temperature. This is because the velocity is limited by one or two steps in the cross-bridge cycle, and their rate constants almost invariably increase with the temperature (Goldman et al., 1987; Zhao and Kawai, 1994). In contrast, tension is determined primarily by the equilibrium constants (Kawai and Zhao, 1993), which may or may not be temperature sensitive. We found that the sliding velocity increased as the temperature was increased from 20 to 35°C (Fig. 8) with $Q_{10} = 3.0$ under the control-activating condition (Fig. 8). This quotient compares to 1.9 (Anson, 1992), 3.7 (calculated from Fig. 5 *B* of Winkelmann et al., 1995), 5.4 (calculated from Fig. 7 of Homsher et al., 1992), and 2.3–2.8 (calculated from the velocity data at 18°C and 40°C of Kato et al., 1999) under similar experimental conditions. Q_{10} in the presence of Pi (3.0) or ADP (2.7) is similar, but there is no previous work carried out in the presence of Pi or ADP.

It is interesting to point out that the sliding velocity decreases in the presence of ADP, much like in the case of the apparent rate constant. This observation can be explained by the mechanism that MgADP binds to the nucleotide binding site on myosin, and it competitively inhibits the ATP hydrolysis rate and work performance (Kawai and Zhao, 1993). It is also interesting to note that the velocity increases in the presence of Pi, as in the case of the apparent rate constant (Kawai and Zhao, 1993). This result may be consistent with the mechanism that Pi binding accelerates an approach to the steady state, and that the velocity behaves much like the apparent rate constant.

Although it may be difficult to carry out experiments on the actomyosin system because of the complexity of the myosin molecule, studying the temperature effect on the kinesin-microtubule system appears to be easier because kinesin is a smaller and simpler molecule than myosin. By using kinesin and microtubule isolated from bovine and porcine brains, respectively, Kawaguchi and Ishiwata (unpublished work) measured temperature dependence of force using optical tweezers. They found that the unitary force (f_1 in Eq. 1) produced by single kinesin molecules was about 7 pN, and this value did not differ in the temperature range between 20 and 35°C. Although the amino acid sequence of the motor domain of myosin and kinesin bears little homology, the tertiary structure of the motor domain bears a striking similarity in terms of the arrangement of α-helices and β-sheets; hence these two proteins are considered to have evolved from a common ancestor (Kull et al., 1998). It is interesting to know that the force that the two motor proteins generate does not differ at different temperatures, and this particular property was maintained throughout the process of evolution. This fact implies that the temperature insensitivity of the single molecular force is a fundamental property of a motor protein, and supports the idea that force is associated with a particular macromolecular architecture.

The results from single-molecule experiments, however, have to be applied to single-fiber experiments with caution, because there is a large difference in the solution composition in the two experimental systems. In particular, single-fiber experiments were carried out at or near physiological ionic strength (180–200 mM), whereas single-molecule experiments were carried out at a low ionic strength (about 50 mM). Because ionic interactions are weakened by increased ionic strength, single-molecule experiments become increasingly more difficult at higher ionic strength. Because the hydrophobic interaction is not affected by the ionic strength, the relative significance of the hydrophobic interaction is pronounced in single-fiber experiments, whereas the relative significance of the ionic interaction is pronounced in single-molecule experiments. As is well known, an increase in temperature facilitates hydrophobic interaction, because this interaction is endothermic (absorbs heat) and accompanies a large entropy increase (Zhao and Kawai, 1994). Similar temperature effect between a single-fiber system and a single-molecule system, in the temperature range of 20–35°C, suggests to us that the hydrophobic interaction may become saturated in this temperature range.

CONCLUSION

We found that force/cross-bridge does not change with temperature in in vitro assay in the temperature range 20–35°C.

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